Method for the Extraction of the Cocaine Metabolite, Benzoylecgonine from Urine using ISOLUTE® HCX and RapidTrace®

Application Note

Introduction

This application note describes for the extraction of the Cocaine metabolite, Benzoylecgonine from urine using ISOLUTE HCX and RapidTrace Workstation.

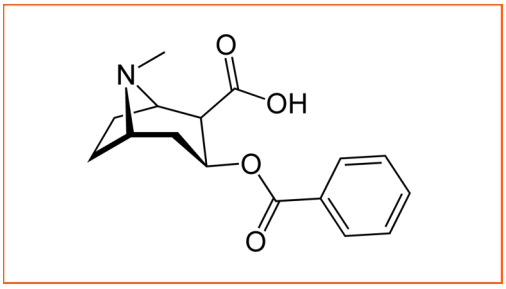

Figure 1. Structure of benzoylecgonine

Cocaine is absorbed and metabolized extremely efficiently and rapidly through the liver which makes it difficult to detect in drugs of abuse testing. When screening for the presence of cocaine in urine it is more appropriate to test for the presence of the major metabolite of cocaine; Benzoylecgonine. Analysis of urine for the presence of Benzoylecgonine gives a better indication of cocaine use than just looking for the main analyte; it allows a profile of use to be constructed and can be used to determine how long ago from the time of testing the cocaine was administered.

Columns

ISOLUTE HCX (3 mL, 130 mg part number 902-0013-B) is a silica-based mixed-mode sorbent optimized to extract drugs of abuse from biological fluids. The combination of non-polar and strong cation retention mechanisms allows for rigorous interference elution steps resulting in extremely clean extracts.

'*The methods offered within this application are meant to represent a starting point and guide for method development and some amendment may be necessary.*'

Analytes

Benzoylecgonine.

The SPE steps were configured schematically using the RapidTrace software as follows:

RapidTrace procedure

	Step	Source	Destination	Volume (mL)	Flow (mL/sec)
1	Condition	Methanol	Waste 2	3.0	0.30
2	Condition	Water	Waste 1	3.0	0.30
3	Condition	H_2PO_4, pH6	Waste 1	3.0	0.30
4	Load	Sample	Waste 1	4.0	0.04
5	Pause		Time = 0.1 min		
6	Purge-Cannula	Water	Cannula	4.0	0.40
7	Rinse	Water	Waste 1	3.0	0.15
8	Rinse	H_2PO_4, pH6	Waste 1	3.0	0.15
9	Rinse	Methanol	Waste 2	3.0	0.15
10	Rinse	Vent	Waste 1	5.0	0.30
11	Collect	Mixed elution	Fraction 1	3.0	0.20
12	Rinse	Mixed elution	Waste 2	2.5	0.20
13	Rinse	Methanol	Waste 2	2.5	0.20
14	Purge-Cannula	Methanol	Cannula	3.0	0.40
15	Purge-Cannula	Water	Cannula	3.0	0.40

Reagent lines

Line 1: Water

Line 2: Methanol

Line 3: 0.1 M Phosphate Buffer, pH6. Prep 1 L by dissolving 1.70 g Na_2HPO_4 and 12.14 g $NAH_2PO_4H_2O$ in 800 mL DI H_2O. Dilute to 1000 mL using DI H_2O and mix. Adjust pH to 6.0 +/- 0.1 with 100 mM monobasic sodium phosphate to lowers pH or 100 mM dibasic sodium phosphate to raise pH. Stable for 1 month when stored at 5 °C.

Line 5: Elution solvent: 800 mL methylene chloride: 200 mL isopropanol: 20 mL concentrated ammonium hydroxide

Biotage recommends TurboVap Workstations post elution to blow down your samples.

Ordering information

Part number	Description	Quantity
902-0013-B	ISOLUTE HCX 3 mL, 130 mg	50
C50000	RapidTrace 3 mL Workstation	1
C50974	Tube Rack (13 x 100 mm Sample Tubes & 12 x 75 Fraction Tubes)	1
C40707	Test Tubes-13 x 100 mm, Uncap	1000
C44651	Test Tubes-12 x 75 mm, Uncap	1000
C52006	RapidTrace Start-Up Kit	1
C52689	RapidTrace Notebook Controller	1

RapidTrace Overview

The RapidTrace has been designed to eliminate sample preparation bottlenecks and it is a modular, highly scalable, automated platform designed for high throughput. Units are available to accommodate 1 mL, 3 mL and 6 mL SPE cartridges. Up to 10 modular units can be connected together and controlled through a simple, easy-to-use software package. The systems are widely used within analytical industry and are ideal both for industrial settings and for efficient SPE method development.

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